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Dry-swabbing/image analysis technique for the pharmaceutical equipment cleaning validation

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Abstract

This paper presents the development of a new technique using the dry-swabbing method for monitoring the contamination of pharmaceutical equipment. Black polyester wipes were used to improve the detection limit of the visual inspection. A standardized method of producing model impurity was used to produce known contamination of the model surface by a variety of compounds ranging from 0 to 500 µg.dm^{-2}. The sample contaminations were dry-swabbed and evaluated by measuring the intensity of contamination using the computer image analysis. The detected intensities of contamination were always proportional to the amount of the impurity applied. The dry-swabbing method has been proven to be at least by one order of magnitude more sensitive than mere visual check.

Keywords: Cleaning validation; dry-swabbing; image analysis; decontamination

1. Introduction

Cleaning validation is a collection of techniques and processes aimed at maintaining the cleanness standards for the pharmaceutical equipment regardless its processing history. The cleaning validation procedures are generally aimed at checking and proving, that the residues of the active pharmaceutical ingredient, remaining at the surface of the machinery are acceptable after finished cleaning; the

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acceptance value being related to the toxicity of API in question. In general, the acceptable level of residual contamination [1, 2] can vary depending on the compound from several hundreds μg per dm^2 of the equipment surface to several μg per dm^2. Unassisted human eye can only identify contamination levels of the several hundred μg per dm^2 magnitude [3], thus in most cases some instrumentation is required to improve sensitivity. The analysis (usually UV or HPLC) of wet swabs from the equipment surface or rinse water represents the industrial standard [4]. Common drawback of both approaches is the necessity to take the sample to laboratory and hence the inevitable delay in continued operation of the production equipment.

This study reports the development of a new technique using the dry-swabbing method for monitoring the contamination of pharmaceutical equipment that is more readily available for routine monitoring of the contamination. The dry-swabbing/image analysis (DSIA) technique employs black polyester wipes for dry-swabbing the equipment surface, so as to transfer all, or at least the representative portion of contamination to the wipe creating visible stain on its surface. Digital photography and computer image analysis can convert the visual information into the numerical intensity, which is proportional to the amount of contamination.

2. Materials and methods

The study involved a variety of tested compounds, including amlodipin, ibuprofen, paracetamol, caffeine, rutin, esculin, losartan etc. and pharmaceutical formulations thereof as a model substances and formulations for investigating the test performance. Those substances were provided by courtesy of Zentiva company (Czech Republic).

2.1. Testing equipment and procedure

The experiments were carried out in laboratory, using plain stainless steel plates, having marked square 1 dm^2 sample areas. Simulated contamination by any of the model contaminants was created by spraying the pre-determined amount of substance solution or suspension over the sample area (fig. 1a). The sprayed volume was maintained constant in order to improve reliability and the contamination level was changed using different concentration of sprayed solution/suspension. Steel plates were then left to dry. This procedure was used to produce a series of stainless steel plates containing known surface contamination by selected model contaminant. Contamination levels ranging from 0 to 500 $\mu g.dm^{-2}$ were generally used. Contaminations over 300 $\mu g.dm^{-2}$ were above the visually detectable limit for most compounds, on the other hand, the contaminations below 150 $\mu g.dm^{-2}$ were not visually detectable.

Then, each sample area was wiped by folded Black Inspection Wiper Class 10.000 (Vestilab SA, Spain) using forceps. The wiping proceeded in a scanning-like manner from left to right edge of the sample area and then again in the top-down direction. The contamination was transferred at least partially onto the wiper, producing a “dry-swab”, containing visible stain, if there was any contamination to be detected. Example of obtained dry-swabs is provided in fig. 2.

The figure shows, that the size and/or intensity of the stain generally increase with the increasing surface contamination. Therefore, it should be possible to use the swabs to quantify the contamination.

Fig. 1. (a) spraying the contaminant solution on model surface area; (b) surface contaminated by 500 μg.dm^{-2} caffeine; (c) surface contaminated by 75 μg.dm^{-2} caffeine

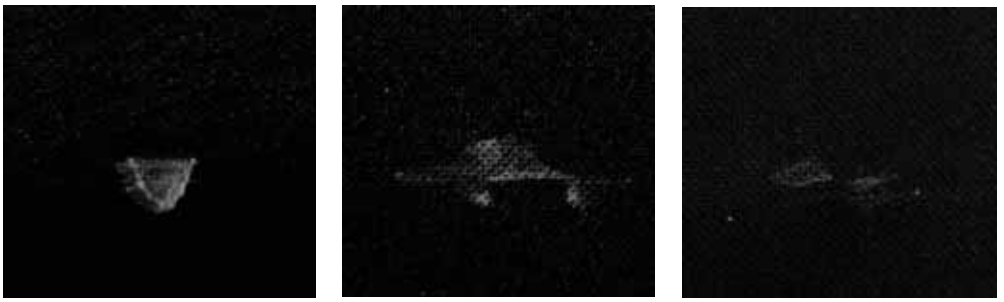

Fig. 2. Dry-swabs of stainless-steel sample area contaminated by Ibalgin® suspension (a) 500 μg.dm^{-2}; (b) 300 μg.dm^{-2}; (c) 75 μg.dm^{-2}

2.2. Dry-swabs image processing and analysis

The shape, size and intensity of the stain depend not only on the contamination of tested surface, but also on the fine details of the swabbing procedure. Among other effects, the intensity of the stain is negatively correlated to its surface area. Hence, it would be difficult to find a reliable relationship between any individual parameter of the stain and the contamination level. Therefore, the obtained swabs were processed by image analysis. Digital images of the obtained swabs were taken using a digital camera. An integral value of luminance was chosen as a representative quantity characterizing the overall intensity of the swab. It was obtained from digital images using the following procedure in Adobe Photoshop software (Adobe, USA):

- The effect of varying light conditions during imaging was corrected by calibration using white and black standards,
- The noise and the wiper texture were suppressed by interpolation,
- The integral value of luminance of the circular area containing the stain I, was determined. The background luminance I_0 was determined over similar circular area of the same size, but on clean unused viper. The difference $I_{net} = I - I_0$ is taken as the net integral intensity of the swab.

The repeatability of the procedure including taking the swab, taking the digital image, and processing it by image analysis varied between RSD = 5 – 10 % for all tested compounds and mixtures.

3. Results and discussion

The dry-sawabs/image analysis technique was tested on a variety of selected APIs and pharmaceutical formulations. Each compound or formulation was tested on five levels of contamination, which were supplemented by blank test. The overview of actual contamination levels for all tested species and obtained results are provided in the tab. 1. The table shows the blanks normally exhibit very low swab intensities. Figures 3 and 4 show the swab intensity being proportional to the contamination level for all tested species, but the slope of the proportion varies from one case to another. The relationship is hence specific for any particular tested material, but it can be approximated by linear dependence, at least for relatively low contamination. Thus, for each material, there can be found a range of contamination, where the relationship between that contamination and the swab intensity is linear. Therefore, within such range the technique can be used to quickly quantify the amount of contamination on tested surface.

Table 1. Swab intensities for contamination of model surface by different contaminants

c, μg.dm^{-2}	500	300	150	75	50	25	5	0
					I × 10^{-3}			
Amlodipin	-	79.2	43.7	24.1	21.1	6.9	-	0.0
Caffeine	226.6	172.5	109.8	57.0	8.7	0.0	-	0.0
Ibuprofen	-	105.0	47.9	25.0	17.3	12.8	-	0.0
Losartan	-	41.2	44.1	55.8	73.3	40.0	-	2.3
Nifuroxazide	-	446.2	249.1	149.7	121.7	128.5	-	9.9
Paracetamol	-	36.5	36.4	20.6	15.1	1.9	-	0.0
Rutin	-	250.3	208.8	191.9	130.1	53.9	-	0.4
Valsartan	-	320.4	147.1	128.9	87.1	-	56.9	0.0
Endiex®	-	386.1	241.9	175.4	145.3	-	71.8	2.4
Ibalgin®	207.3	236.7	87.1	42.2	32.0	0.0	-	0.2
Lozap H®	-	216.2	122.2	102.8	92.6	-	40.3	3.9
Valzap®	-	224.1	108.7	71.2	22.9	-	5.9	2.4

The figs. 3 – 4 and data in tab. 1 show certain relationship between active ingredient and respective formulation. However the relationship has a long way to go for being universally valid rule, so that relevant formulation has to be used for method calibration for each specific compound or mixture, whatever is appropriate for the surface being examined.

The intensity-contamination relationships are linear in wide range for some samples (fig. 3) or they may deviate from the linearity at higher concentrations (fig. 4). The deviation can be due to the stain oversaturation as it is the case for Ibalgin or due to the unfavorable optical properties of tested material in case of losartan.

The observations above were summarized for all tested compounds in tab. 2, showing the conservative estimate of the detection limit (LOD) and the linear range of quantification (ROQ). The LOD estimate is expressed as the lowest contamination that was actually tested and produced swab intensity 3 times higher than that of blank sample. ROQ reports the range from LOD up to the limit of calibration curve linearity.

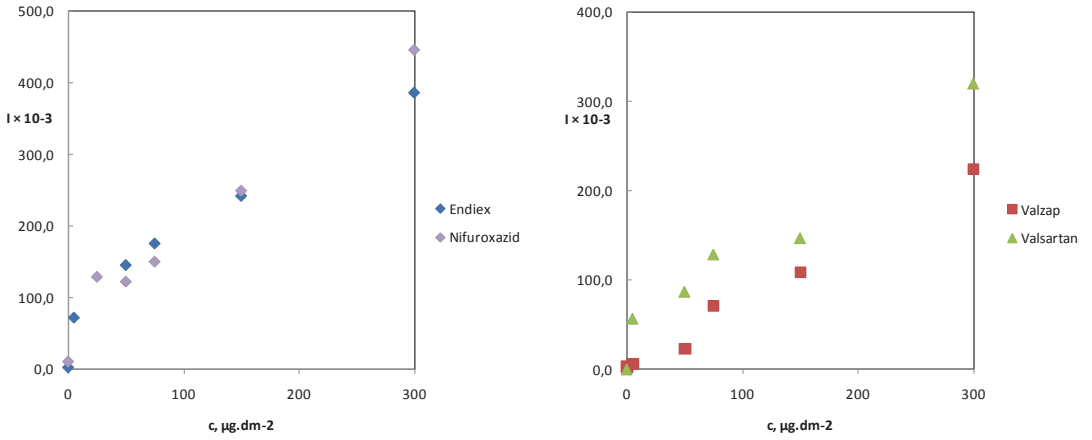

Fig. 3. Relationship between the swab intensity and contamination of the sample surface by (a) Endiex® formulation and nifuroxazide; (b) Valzap® formulation and valsartan

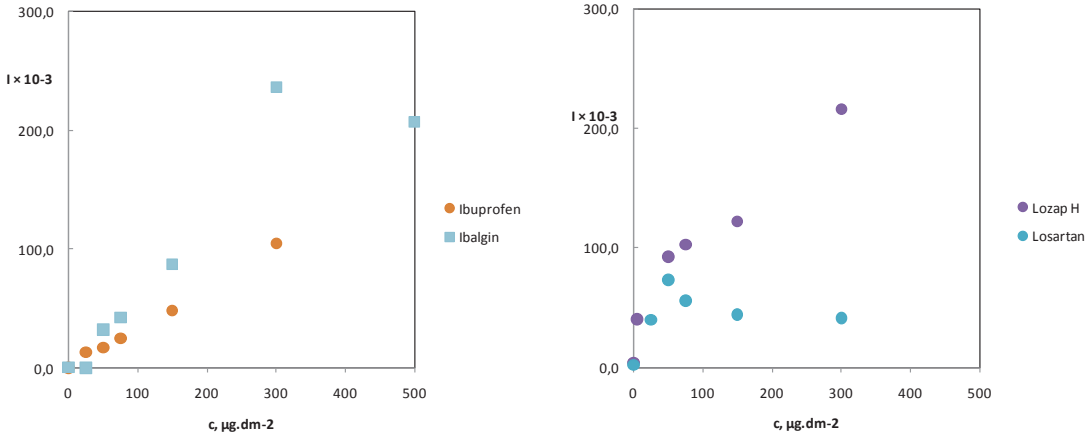

Fig. 4. Relationship between the swab intensity and contamination of the sample surface by (a) Ibalgin® formulation and ibuprofen; (b) Lozap H® formulation and losartan

Table 2. Limits of detection and ranges of quantification of surface contamination by dry-swabbing method for variety of pharmaceutical ingredients/formulation

c, $\mu.dm^{-2}$	LOD	ROQ
Amlodipin	< 25	25 - 300
Caffeine	25 - 50	50 - 300
Ibuprofen	< 25	25 - 300
Losartan	< 25	25 - 75
Nifuroxazid	< 25	25 - 300
Paracetamol	< 25	25 - 150
Rutin	< 25	25 - 100
Valsartan	< 5	5 - 300
Endiex®	< 5	5 - 300
Ibalgin®	25 - 50	50 - 300
Lozap H®	< 5	5 - 300
Valzap®	5 - 50	50 - 300

4. Conclusions

The developed technique of dry-swabs/image analysis was proved useful for quick determination of surface contamination by pharmaceutical formulation. All tested substances and formulations exhibited statistically significant intensity-contamination relationship. The detected intensities of contamination were always proportional to the amount of the impurity applied. Even the smallest test contamination of 25 $\mu g.dm^{-2}$, left apparent contamination stains on the swab, while the zero-level sample showed no visible trace of contamination. Most relationships exhibited very god linearity in the contamination ranges of interest (25 – 250 $\mu g.dm^{-2}$). For higher contamination, the linearity was poor, due to stains over-saturation, but those contamination levels are so high that they are detectable by unassisted eye. Distinct differences among test compounds were observed, thus “per substance” calibration must be performed to obtain relevant results.

It can be concluded that for practical application of this technique, it would be best to prepare a sample of swabs in laboratory environment first. These standardized swabs could be afterwards compared visually with swabs applied in operating conditions. This would enable the final users to make rapid and reliable estimates of the intensity of contamination. The dry-swabbing method has been proven to be at least by one order of magnitude more sensitive than simple visual check.

Acknowledgements

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